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Synthesis and biological evaluation of 4-piperidinecarboxylate and 4-piperidinecyanide derivatives for T-type calcium channel blockers

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ABSTRACT

To obtain selective and potent inhibitor for T-type calcium channel by ligand based drug design, 4-piperidinecarboxylate and 4-piperidinecyanide derivatives were prepared and evaluated for in vitro and in vivo activity against α_{1G} calcium channel. Among them, several compounds showed good T-type calcium channel inhibitory activity and minimal off-target activity over hERG channel (% inhibition at 10 μM = 61.85–71.99, hERG channel IC_{50} = 1.57 ± 0.14–4.98 ± 0.36 μM). Selected compound **31a** was evaluated on SNL model of neuropathic pain and showed inhibitory effect on mechanical allodynia.

Calcium ion performs a significant role as an intracellular second messenger and many physiological functions such as cell contraction, hormone secretion, neurotransmission, gene expression, cell growth and division.[1] Voltage-dependent calcium channels (VDCC) are main calcium entry pathway in the cellular membrane by change of membrane potential and play a significant physiological role in excitability cells. According to electrophysiological and pharmacological criteria, voltage-dependent calcium channels can be divided into high voltage-activated (L, N, P, Q, R-type) and low voltage-activated (T-type).[2] But, on the basis of molecular cloning of the main pore-forming 10 different α_1 subunits, they have been classified in three families: $Ca_V1.1$–1.4 (L-type), $Ca_V2.1$–2.3 (N, P, Q/R-type), $Ca_V3.1$–3.3 (T-type). T-type calcium channels are found in CNS, peripheral tissues, heart, smooth muscle, kidney and endocrine tissue, and they have been proposed to be involved in cardiac pace-making, blood pressure regulation,[3] and the secretion of hormones including aldosterone and insulin.[4] Furthermore, T-type calcium channels are identified in the thalamus and cortex where they have a crucial role in the integration of neuronal firing for thalamocortical signaling.[5] Therefore, T-type calcium channels are considered to participate in a variety of human diseases including insomnia,[6] neuropathic pain, epilepsy, certain types of cancer, cardiac arrhythmia and hypertension.[7,8]

Mibefradil (**1**), which was developed for the treatment of hypertension and angina pectoris, has a 10- to 30-fold selectivity for T-type over L-type channel, but was withdrawn from the market due to drug–drug interaction.[9,10] Despite T-type calcium channels has known as novel therapeutic targets, no potent and selective T-type calcium channel blocker is available for clinical use until now. Although dihydropyridine (DHP) compound, penfluridol (**2**), was reported to have blocking activity, their clinical usage is limited because of low efficacy and selectivity.[11,12] Recently, T-type channel blockers with a piperidine scaffold were reported to have good potency with minimal effects on L-type and hERG channels and showed in vivo efficacy in epilepsy.[14a,15] Based on this results, 1,4-substituted piperidine derivatives (**3**, Fig. 1) were designed and prepared for T-type calcium channel blockers using 3D ligand based pharmacophore model by common feature generation approach (HipHop) implemented in CATALYST program.[13]

From the pharmacophore mapping data and our previous study, 3,5-disubstituted phenyl and pyrazole moiety (R^3 groups) are beneficial for potency, and *N*-alkyl group (R^1) of the piperidine ring were favorable for activity. Also in the point of R^2 position, electron withdrawing group (EWG), such as cayno and methyl ester group, plays a crucial role on reducing the undesirable side effect of hERG channel by lowering of the basicity of piperidine ring.[14a,b] (**3**, Fig. 1) To obtain focused library with a suggested scaffold, we have carried out the next reactions.

N-Boc-4-cyanopiperidine was prepared from commercially available 4-cyanopiperidine (**4**), which was converted to the ethyl ester (**5**) by treatment with LDA and ethyl chloroformate in THF at −78 °C. The cyano group of **5** underwent reduction with PtO_2 in AcOH to provide **6**. The resulting amino group was protected with benzyloxycarbonyl (Cbz) group to give **7**. The ethyl ester group of **7** was hydrolyzed with NaOH in THF–H_2O under reflux condition to provide acid compound, which was coupled with ammonia by using EDCI and HOBt to give amide compound **8**. Dehydration of

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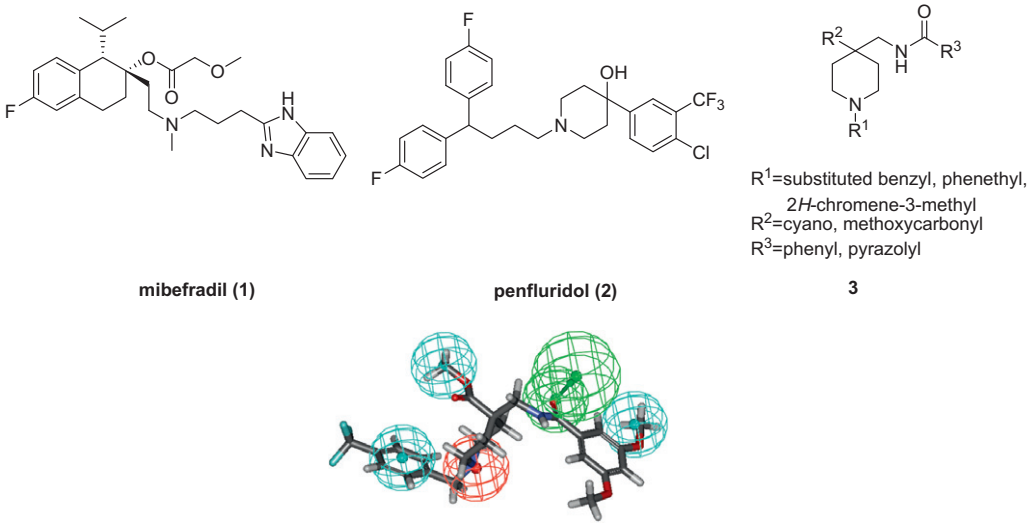


Figure 1. T-type calcium channel blockers and 5-feature pharmacophore mapping of **28f** (cyan: hydrophobic, green: hydrogen bond acceptor, red: positive ionizable).

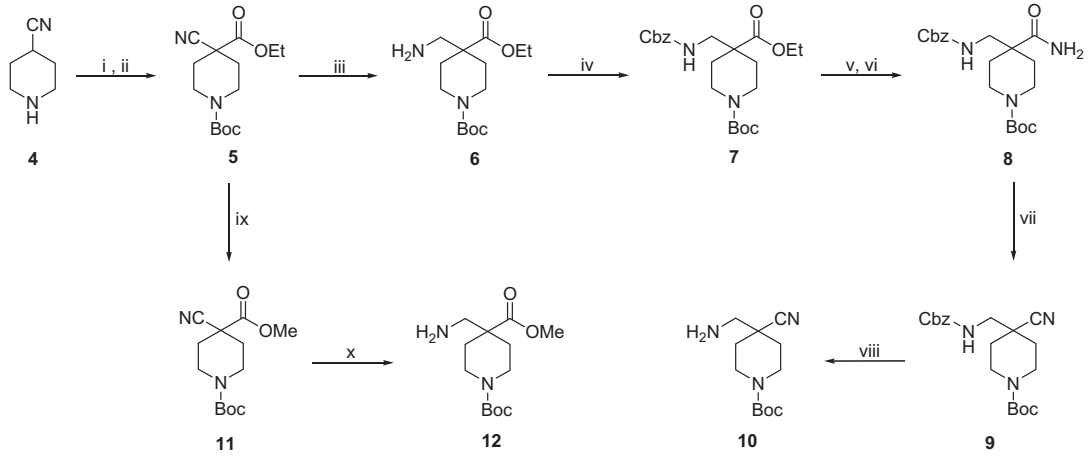
the amide group of **8** with TFAA in the presence pyridine at 0 °C afforded **9**. Cbz protecting group of **9** was removed to the free amine (**10**) with 10% Pd/C in MeOH. The ethyl ester (**5**) was converted to methyl ester (**11**) with NaOMe in methanol. The cyano group of **11** underwent reduction with PtO_2 in AcOH to provide **12** (Scheme 1).

To obtain the R^3 substituents, we purchased benzoic acid derivatives, but pyrazole carboxylic acid was prepared as follows. 2,4-Dioxoheptanoate (**14**), which was synthesized from 4-methyl-2-pentanone (**13**) and diethyl oxalate with NaOEt, was converted into 1*H*-pyrazole **15** by the addition of phenyl hydrazine in EtOH. And the ethyl ester group of **15** was hydrolyzed with NaOH in THF–H_2O under reflux condition to provide corresponding acid compound **16** (Scheme 2).

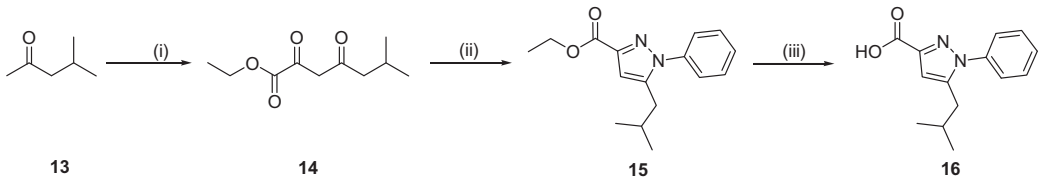
For the synthesis of R^2 substituent, we employed commercially available benzyl halide and aldehyde except chromene moiety which was easily synthesized by next reactions. Base-catalyzed condensation reaction of salicylaldehyde (**17**) and 3-methyl-2-butenal with Na_2CO_3 in dioxane/H_2O = 3:1 afforded compound **18**, which was treated with DIBAL-H to provide primary alcohol **19**. Then, chlorination of **19** with $SOCl_2$ in CH_2Cl_2 gave compound **20** (Scheme 3).

The aminomethyl group of compounds **10, 12** were directly coupled with 3,5-dimethoxybenzoyl chloride and 3,5-dichlorobenzoyl chloride in CH_2Cl_2 to provide the amide compounds and then the Boc group was removed to the free amine with 2 N HCl in EtOAc to provide **21–24**. Then, alkylation of secondary amine of piperidine compounds **21–24** with benzyl halides **a–g** with NaI, TEA in CH_3CN under reflux condition afforded **26–29a–g**. Also, compounds **21–24** were coupled with aldehyde through reductive amination using $NaBH(OAc)_3$ to give **26–29h** (Scheme 4). Compounds **30a–h** were synthesized by the same procedure as shown in Scheme 4 (Scheme 5).

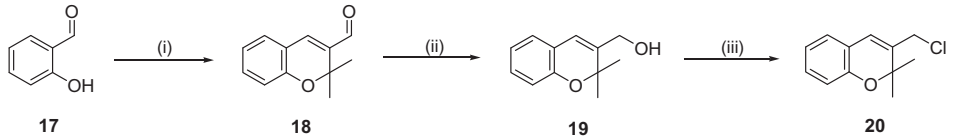
In vitro screening of all the synthesized compounds was performed with HEK293 cells expressed T-type calcium channels (α_{1G}) at 10 μM concentration.[16] From this preliminary data, the selected compounds which showed above 50% blocking effect against T-type calcium channel were evaluated through hERG channels by whole-cell patch clamp method[17] to get the selective lead compound over T-type/hERG channel. Blockade of the hERG channel can lead to a heart rhythm disorder known as long QT syndrome, characterized by prolonged action potential of ventricular muscle.[18] In vitro biological data of the compounds were shown in Tables 1–3 and mibefradil was used as reference compound.[19]



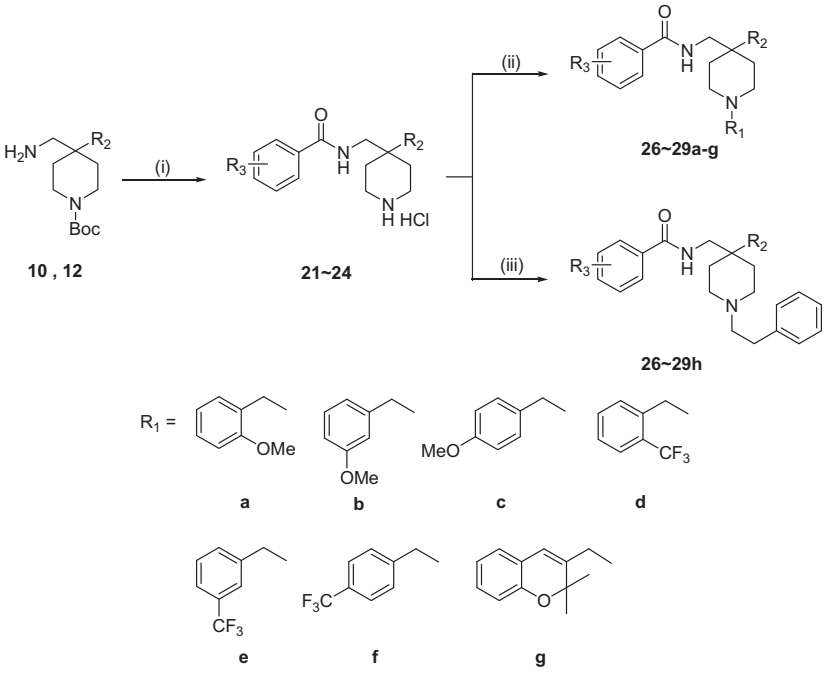
Scheme 1. Reagents and conditions: (i) Boc_2O, TEA, CH_2Cl_2, 0 °C to rt, 96%; (ii) ethyl chloroformate, LDA, THF, −78 °C to rt, 65%; (iii) H_2, PtO_2, AcOH, rt, 80%; (iv) benzyl chloroformate, $NaHCO_3$, CH_2Cl_2, 0 °C to rt, 92%; (v) NaOH, EtOH/H_2O (1:1), reflux, 93%; (vi) EDCI, HOBt, DMF, rt, then 35% NH_4OH, 94%; (vii) TFAA, pyridine, CH_2Cl_2, 0 °C to rt, 77%; (viii) H_2, Pd/C, MeOH, rt, 83%; (ix) NaOMe, MeOH, rt, 95%; (x) H_2, PtO_2, AcOH, rt, 73%.



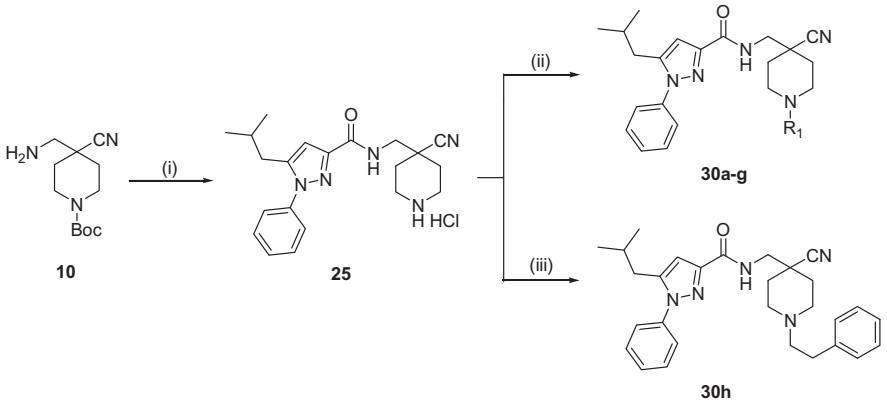
Scheme 2. Reagents and conditions: (i) diethyl oxalate, NaOEt, EtOH, 0 °C to rt, 81%; (ii) phenyl hydrazine, EtOH, 0 °C to rt, 70%; (iii) NaOH, EtOH/H_2O (1:1), reflux, 87%.



Scheme 3. Reagents and conditions: (i) 3-methyl-2-butenal, Na_2CO_3, dioxane/H_2O (3:1), 55 °C, sonication, 64%; (ii) DIBAL-H, CH_2Cl_2, −78 °C, 70%; (iii) $SOCl_2$, CH_2Cl_2, rt, 87%.

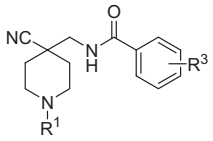


Scheme 4. Reagents and conditions: (i) 3,5-disubstituted benzoyl chloride, TEA, CH_2Cl_2, 0 °C to rt, then 2 N HCl in EtOAc, 68–82%; (ii) R_1 halides, TEA, NaI, CH_3CN, 80 °C, 25–89%; (iii) phenylacetaldehyde, $NaBH(OAc)_3$ TEA, $ClCH_2CH_2Cl$, rt, 41–64%.



Scheme 5. Reagents and conditions: (i) compound **16**, EDCI, HOBt, TEA, DMF, rt, then 2 N HCl in EtOAc; (ii) R_1 halides, TEA, NaI, CH_3CN, 80 °C, 41–69%; (iii) phenylacetaldehyde, $NaBH(OAc)_3$ TEA, $ClCH_2CH_2Cl$, rt, 65%.

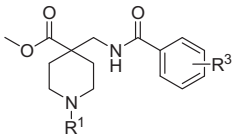
Table 1
In vitro activities of synthesized compound (**1**)



Compound	R^1	R^3	% Inhibition[a] (10 μM)	hERG[b] IC_{50} (μM)
26a	2-Methoxybenzyl	3,5-Dimethoxy	50.93	0.92 ± 0.04
26b	3-Methoxybenzyl	3,5-Dimethoxy	60.41	1.50 ± 0.23
26c	4-Methoxybenzyl	3,5-Dimethoxy	53.03	3.21 ± 0.18
26d	2-(Trifluoromethyl)benzyl	3,5-Dimethoxy	45.44	4.76 ± 0.72
26e	3-(Trifluoromethyl)benzyl	3,5-Dimethoxy	15.75	NT
26f	4-(Trifluoromethyl)benzyl	3,5-Dimethoxy	13.88	NT
26g	2,2-Dimethyl-2*H*-chromenyl	3,5-Dimethoxy	44.30	4.73 ± 0.73
26h	Phenethyl	3,5-Dimethoxy	61.85	1.57 ± 0.14
27a	2-Methoxybenzyl	3,5-Dichloro	19.31	NT
27b	3-Methoxybenzyl	3,5-Dichloro	8.33	NT
27c	4-Methoxybenzyl	3,5-Dichloro	16.11	NT
27d	2-(Trifluoromethyl)benzyl	3,5-Dichloro	9.99	NT
27e	3-(Trifluoromethyl)benzyl	3,5-Dichloro	8.76	NT
27f	4-(Trifluoromethyl)benzyl	3,5-Dichloro	8.70	NT
27g	2,2-Dimethyl-2*H*-chromenyl	3,5-Dichloro	13.57	NT
27h	Phenethyl	3,5-Dichloro	71.79	0.36 ± 0.06
Mibefradil			78.95	1.34 ± 0.49

[a] % Inhibition was obtained at 10 μM.
[b] IC_{50} value (±SE) was determined from dose–response curve ($n = 3$).

Table 2
In vitro activities of synthesized compound (**2**)

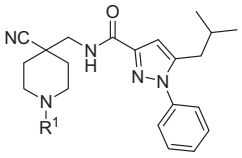


Compound	R^1	R^3	% Inhibition[a] (10 μM)	hERG[b] IC_{50}(μM)
28a	2-Methoxybenzyl	3,5-Dimethoxy	46.78	1.90 ± 0.06
28b	3-Methoxybenzyl	3,5-Dimethoxy	56.16	0.31 ± 0.01
28c	4-Methoxybenzyl	3,5-Dimethoxy	58.98	0.48 ± 0.05
28d	2-(Trifluoromethyl)benzyl	3,5-Dimethoxy	63.57	4.98 ± 0.77
28e	3-(Trifluoromethyl)benzyl	3,5-Dimethoxy	71.99	2.80 ± 0.36
28f	4-(Trifluoromethyl)benzyl	3,5-Dimethoxy	64.29	2.35 ± 0.45
28g	2,2-Dimethyl-2*H*-chromenyl	3,5-Dimethoxy	57.99	1.29 ± 0.15
28h	Phenethyl	3,5-Dimethoxy	64.96	NT
29a	2-Methoxybenzyl	3,5-Dichloro	68.46	0.16 ± 0.03
29b	3-Methoxybenzyl	3,5-Dichloro	73.03	0.36 ± 0.07
29c	4-Methoxybenzyl	3,5-Dichloro	74.46	0.13 ± 0.01
29d	2-(Trifluoromethyl)benzyl	3,5-Dichloro	44.15	0.46 ± 0.01
29e	3-(Trifluoromethyl)benzyl	3,5-Dichloro	71.96	0.89 ± 0.11
29f	4-(Trifluoromethyl)benzyl	3,5-Dichloro	43.30	0.29 ± 0.05
29g	2,2-Dimethyl-2*H*-chromenyl	3,5-Dichloro	33.93	13.83 ± 2.24
29h	Phenethyl	3,5-Dichloro	77.18	NT
Mibefradil			78.95	1.34 ± 0.49

[a] % Inhibition was obtained at 10 μM.
[b] IC_{50} value (±SE) was determined from dose–response curve ($n = 3$).

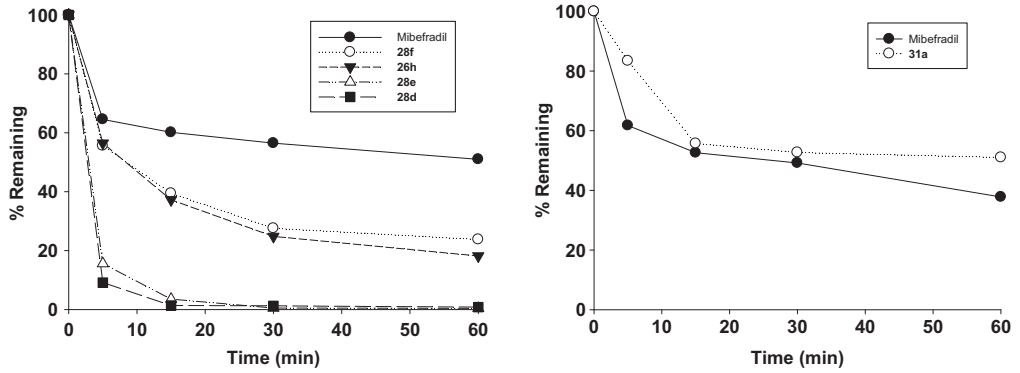
As shown in Tables 1–3 on the N-1 position of piperidine (R^1), the order of activity was generally phenethyl > benzyl > chromenyl. The methyl ester derivatives on C-4 position of piperidine ring showed better inhibitory activity than corresponding cyano derivatives, especially in the case of the substituent of benzoyl amide (R^3) is di-chloro (Table 2). Contrary to our expectation based on pharmacophore mapping study, there is less significant improvement with inhibitory activity of pyrazole amide substituent compared to benzoyl moiety. The inhibitory activity of pyrazole amide is similar to that of benzoyl amide. Summing up every preliminary in vitro assay data, four compounds **26h** and **28d–f** were chosen for the in vitro stability test in human hepatic microsome.[20] According to the metabolic stability result of **26h** and **28d–f,** they were very unstable (Fig. 2a). The main reason was supposed to be the hydrolysis of phenethyl and methyl ester group. Thus, methyl ester group of **28f** was converted to carboxylic acid group (**31a**) with NaOH in THF–H_2O under reflux condition, and then the in vitro stability was re-evaluated. As expected, compound **31a** was more stable than parent compound **28f** and mibefradil (Fig. 2b).

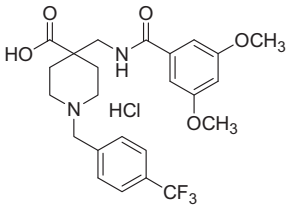
Table 3
In vitro activities of synthesized compound (**3**)



Compound	R^1	% Inhibition[a] (10 μM)	hERG[b] IC_{50}(μM)
30a	2-Methoxybenzyl	50.93	0.92 ± 0.04
30b	3-Methoxybenzyl	60.41	1.50 ± 0.23
30c	4-Methoxybenzyl	53.03	3.21 ± 0.18
30d	2-(Trifluoromethyl)benzyl	45.44	4.76 ± 0.72
30e	3-(Trifluoromethyl)benzyl	15.75	NT
30f	4-(Trifluoromethyl)benzyl	13.88	NT
30g	2,2-Dimethyl-2*H*-chromenyl	44.30	4.73 ± 0.73
30h	Phenethyl	61.85	1.57 ± 0.14
Mibefradil		78.95	1.34 ± 0.49

[a] % Inhibition was obtained at 10 μM.
[b] IC_{50} value (±SE) was determined from dose–response curve (n = 3).





31a

Figure 2. In vitro metabolic stability of **26h**, **28d**–**f**, and **31a** in human hepatic microsomes. Human liver microsomes (0.5 mg/ml) and **26h**, **28d**–**f**, and **31a** (1 μM) in potassium phosphate buffer (0.1 M, pH 7.4) were incubated at 37 °C. Reactions were initiated by the addition of β-NADPH (1.2 mM) and continued at 37 °C. After incubation for 0, 5, 15, 30, and 60 min, the reaction was stopped by addition of acetonitrile with internal standard. The samples were analyzed using LC–MS/MS. Mibefradil was used as a reference standard. The results are expressed as the percentage of drug remaining after incubation. Data represent the mean of duplicated experiments.

Compound **31a** showed similar activity against T-type calcium channel and better activity over hERG channel (IC_{50} of α_{1G} = 7.31 ± 0.96, IC_{50} of hERG = 12.08 ± 0.76) compared to **28f** (IC_{50} of α_{1G} = 4.18 ± 0.51, IC_{50} of hERG = 2.35 ± 0.45). T-type calcium channel blockers seem to be promising therapeutic agents for the treatment of neuropathic pain, thus **31a** was stepped into the in vivo test for neuropathic pain model. Behavioral test for neuropathic pain according to the spinal nerve ligation model[21] (Chung's model) was done with 20 rats. After 14 days of surgical manipulation, the experiment for effect on allodynia of **31a** was performed. Compound **31a** showed suppression effect toward mechanical allodynia and little inhibitory effect on cold allodynia (Fig. 3), however, **31a** seems to have somewhat weak mechanical allodynia effect in comparison with gabapentin.

In summary, the piperidine derivatives designed and prepared as T-type calcium channel blockers through previous SAR and pharmacophore mapping study. Compound **31a** was evaluated on a rat model of neuropathic pain and it reduced mechanical allodynia by oral administration. Further assay and optimization on the piperidine derivatives may lead to development of potent and selective T-type calcium channel blockers for the treatment of disorders related to this channel.

Acknowledgments

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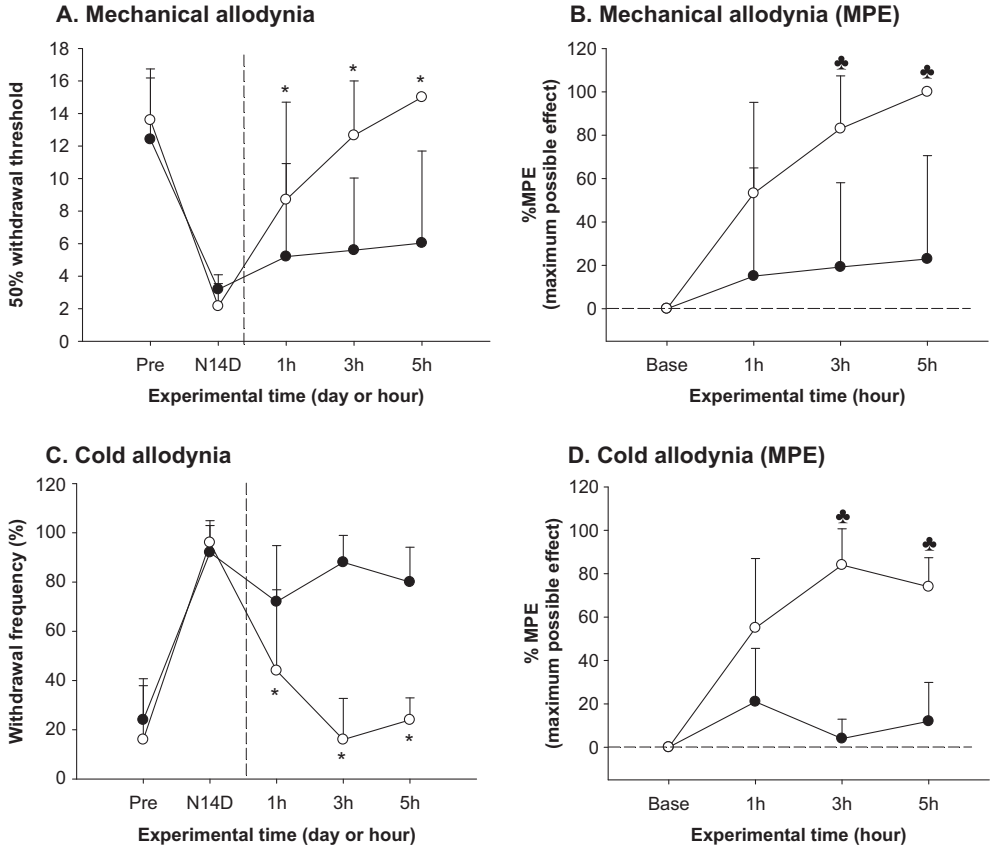

Figure 3. Effect on mechanical allodynia (A and B) and cold allodynia (C and D) after oral administration of gabapentin (○, 100 mg/kg, $n = 5$) and **31a** (●, 100 mg/kg, $n = 5$) to neuropathic pain induced rats. Experimental time expressed as D for days after neuropathic injury (N) and h for hours after gabapentin or **31a** administration, $^{*}P < 0.05$ (gabapentin), ♣$P < 0.05$ gabapentin versus **31a** (unpaired *t*-test).

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16. (a) Kim, T.; Choi, J.; Kim, S.; Kwon, O.; Nah, S. Y.; Han, Y. S.; Rhim, H. *Biochem. Biophy. Res. Commun.* **2004**, *324*, 401; (b) HEK293 cells which stably express both α_{1G} and Kir2.1 subunits were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum, penicillin (100 U/mL), streptomycin (100 μg/mL), geneticin (500 μg/mL), and puromycin (1 μg/mL) at 37 °C in a humid atmosphere of 5% CO_2 and 95% air. Cells were seeded into 96-well black wall clear bottom plates at a density of 4×104 cells/well and were used the next day for high-throughput screening (HTS) FDSS6000 assay. For FDSS6000 assay, cells were incubated for 60 min at room temperature with 5 μM fluo3/AM and 0.001% Pluronic F-127 in a Hepes-buffered solution composed of (in mM): 115 NaCl, 5.4 KCl, 0.8 $MgCl_2$, 1.8 $CaCl_2$, 20 Hepes, and 13.8 glucose (pH 7.4). During the fluorescence-based FDSS6000 assay, α_{1G} T-type Ca^{2+} channels were activated using high concentration of KCl (70 mM) in 10 mM $CaCl_2$ contained Hepes-buffered solution and the increase in $[Ca^{2+}]_i$ by KCl-induced depolarization was detected. During the whole procedure, cells were washed using the BIO-TEK 96-well washer. All data were collected and analyzed using FDSS6000 and related software (Hamamatsu, Japan).
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